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Submission date: 29-May-2019 09:49AM (UTC+0800)
Submission ID: 1137182702
File name: 16._Widji_Soeratri.doc (85.5K)
Word count: 1369
Character count: 7275

The Influence of Olive Oil Addition on Increasing of Arbutin Penetration in the *Carbomer-940's* Gel (Observation on Inhibition of Enzyme Tyrosinase Activity)

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Abstract

The aim of this study was to investigate the influence of olive oil addition on the increasing of arbutin penetrations in the *Carbomer-940's* gel; it was observed on inhibition of enzyme tyrosinase activity. High hydrophilicity of arbutin (log P value, -1.35) makes it difficult to permeate through the skin and reach site of action. Olive oil addition was expected to increase the arbutin penetrations. Percent inhibition of tyrosinase by arbutin was determined by observing absorption value of Dopachrome (in intermediate product of melanin formation) using spectrophotometer. The result of this study, percent inhibition of the formula control, 1, 2 and 3 were 21.79; 24.24; 21.79 and 35.60% respectively. The processing data was using the statistic programmed of SPSS 12.0 with one way ANOVA method obtained the result there was significant difference from percent inhibition in one of formula. Conclusion from this study was olive oil addition 7% could increase of arbutin penetrations and increase the inhibition of enzyme tyrosinase activity.

Keyword: Arbutin, Penetrations, Olive oil, Enzyme tyrosinase, *Carbomer-940's*, inhibition

Introduction

Arbutin is mostly used as lightening agent which was very hydrophilic with log P value -1.35; make it difficult to penetrate through the skin. Enhancer can be adding to increase penetration rate. Olive oil (3, 5 and 7%) addition was expected to increase the arbutin penetrations. Olive oil contains oleic acid (83.5%), a substance that's capable of interacting and modifying the lipid bilayer of stratum corneum to increase the lipophilicity of a substance. Its ability as penetration enhancer in local anesthetic agent has been proofed by Sarma and Fisher (Sarma, 1993).

Among several variant skin lightening preparations, gel gives us a cool sensation, not sticky, elegant, and smooth and easy to be washed from the skin. A synthetic gelling agent like *carbomer* 940 usually requires only a small amount of them to produce a gel with good consistency compared to other types of gelling agent.

The aim of this study was to investigate the influence of olive oil (3, 5, and 7% w/w) addition on the arbutin (3% w/w) penetrations in the Carbomer-940 gel base through the modified lipid membrane. It was observed on inhibition of enzyme tyrosinase activity.

Methodology

Preparation of skin lightening gel containing arbutin and olive oil

The arbutin in Carbomer-940 gel base formulas as lightening product was shown in table 1.

Characteristics determination of skin lightening gel

The characteristic of preparation were determined include: organoleptic test visually. While the determination of pH and spread ability is done in 2 days after the formula were made by using a digital pH meter Schott CG 842. and a spreading-ability apparatus.

Table 1: Formula used in research

Material	Consentration (% w/w)				
	Base	Control	F1	F2	F3
Arbutin	-	3	3	3	3
Olive oil	-	-	3	5	7
Carbomer 940	1	1	1	1	1
TEA	1	1	1	1	1
Propylene glycol	20	20	20	20	20
Methyl-parabene	0.15	0.15	0.15	0.15	0.15
Propyl-parabene	0.05	0.05	0.05	0.05	0.05
Na EDTA	0.05	0.05	0.05	0.05	0.05
BHT	0.05	0.05	0.05	0.05	0.05
Tween 80	0.5	0.5	0.5	0.5	0.5
Water up to	100	100	100	100	100

Determination of enzyme tyrosinase activity

L-tyrosine solution 0.5 ml added with 3.0 ml sample solution that collected from compartment receptor after 360 minutes penetrated through Millipore membrane which was impregnated with isopropyl-myristate. The mixture was oxygenized 5 minutes then added with 1.0 ml tyrosinase solution. After incubated for 10 minutes at 25ºC the mixture was inactivated with 0.5 ml TCA solution and then the absorption value measured at maximum wavelength of dophacrome (Avanti, 2003).

The evaluation of inhibition of enzyme tyrosinase activity

The inhibition of enzyme tyrosinase activity was performed as inhibition percent, which found from calculation of absorption value per second enzymatic reaction with inhibitor, compared with absorption value per second enzymatic reaction without inhibitor, using the following equation (Luanratana and Gritsadapong, 2005):

$$\text{inhibition}\,(\%) = 100 - \frac{(Ax100)}{B}$$

Whereas:
A = absorption value (A/second) at dophacrome λ maximum with inhibitor
B = absorption value (A/second) at dophacrome λ maximum without inhibitor

The data (inhibition %) were analyzed with ANOVA one way method ($p<0.05$).

Results and Discussions

The pH of arbutin gels shows in table 2 indicated that 3, 5, and 7% w/w concentration of olive oil not influenced the pH value of arbutin gels. Based on the pH data was known that all formulas have suitable pH (5.84 – 6.53) for skin around 4.0 – 6.8 (Zulkarnain, 2003).

Figure 1 shows the spreading profile of arbutin gel preparation, and spreading-capasity (spreadingdiameter) of arbutin gels at 45 gram ballast shows in table 3. The ANOVA one way test of spreading-capacity found the value of $F_{calculation}$ (196.533) > F_{table} (4.07). Based on the HSD test can concluded that addition 7% w/w olive oil decreased the spreading-capacity of arbutin gel preparation. Spreading-ability was the slope of linier-regression between spreading-diameter (mm) and ballast weight (gram), its shows in table 4. The slope value from its formulas was tested by ANOVA one way method, it's found that the value of $F_{calculation}$ (23.669) < F_{table} (4.07). From HSD test can concluded that addition

olive oil decreased the spreading-ability of arbutin gel preparation by increasing concentration of olive oil 3, 5 and 7% w/w.

Table 2. The arbutin gel pH values

Formula	pH	
	Mean ± SD	**%CV**
Base	6.00 ± 0.01	0.17
Control	6.16 ± 0.11	1.78
Formula 1	5.84 ± 0.08	1.37
Formula 2	6.53 ± 0.05	0.76
Formula 3	6.45 ± 0.02	0.31

The arbutin effectivity as lightening agent calculated as inhibition percent (%) of enzyme tyrosinase activity. The result of arbutin inhibition percent (%) with enhancer olive oil in carbomer gels shows in table 5.

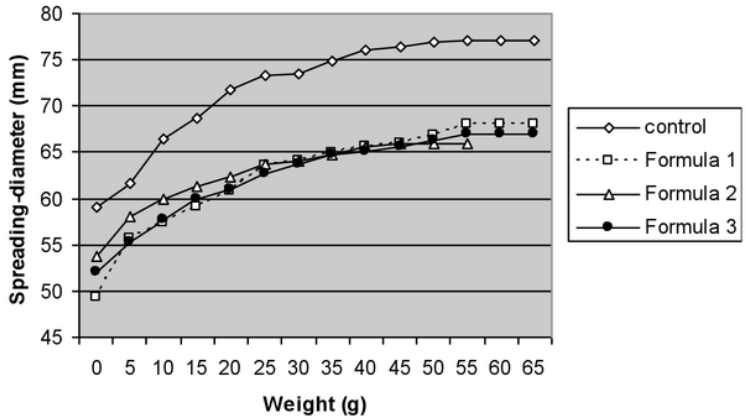

Figure 1. The spreading profile of arbutin gel preparation.

The ANOVA one way test of the arbutin effectivity in carbomer gel formulas found the value of $F_{calculation}$ (29.739) > F_{table} (4.07), and from the HSD test was found inhibition percent of control = formula 1 = formula 2 < formula 3.

Table 3. Spreading-capacity arbutin gel at 45 gram ballast weight

Formula	Spreading diameter mean ± SD (mm)
Control	76.33 ± 0.58
Formula 1	66.00 ± 0.00
Formula 2	66.00 ± 1.00
Formula 3	65.67 ± 0.58

Table 4. Spreading-ability of arbutin gel

Formula	Average slope ± SD (g/mm)
Control	0.3473 ± 0.02
Formula 1	0.2965 ± 0.01
Formula 2	0.2550 ± 0.01
Formula 3	0.2497 ± 0.01

Addition 3 and 5% w/w enhancer olive oil did not influence arbutin effectivity it can be caused by decreasing arbutin release from the more viscous bases. In addition of 7 % w/w olive oil was known that it increased arbutin penetration, showed by increasing inhibition percent of tyrosinase activity. It more is caused increasing concentration of arbutin in water phase, replacing water by olive oil in the formula.

Table5. Arbutin effectivity (% inhibition) in skin lightening gel

Formula	% Inhibition	
	Average ± SD	% CV
Control	21.79 ± 1.65	7.57
Formula 1	24.24 ± 2.57	10.60
Formula 2	21.79 ± 1.65	7.57
Formula 3	35.60 ± 2.35	6.60

Conclusion

The addition of arbutin and olive oil affect the physical appearance (organoleptic and consistency) of skin lightening product as well as its spreading ability. Addition 7 % w/w olive oil increase arbutin effectivity by increased the inhibition percent value of enzyme tyrosinase activity in the skin lightening formula which was formulated in carbomer 940 gel base.

Acknowledgement

This study was supported financially by Project Grant Faculty of Pharmacy. Airlangga University.

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